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MULTI-COLUMN LIQUID CHROMATOGRAPHY

III. NEW TECHNIQUES FOR MULTI-COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

New techniques for multi-column chromatography include a new system using nitrogen pressure and a system using a single pump to drive an eluent simultaneously through a number of chromatographic columns.

INTRODUCTION

Some years ago we published a system for simultaneous twenty-five-column liquid chromatography[1]. Working with the system we found that we could significantly improve the design by replacing the complex mixing cylinder having twenty-five individual outlets and connected to a panel with twenty-five valves with a cylinder with two outlets or a single outlet and a distributor with twenty-five valved outlets.

The advantage of this design is not only that a considerable simplification of the system allows the use of fewer parts but also that either pumps or nitrogen under pressure can be used for driving a solvent or gradient through a number of chromatographic columns simultaneously and that a single pump of adequate capacity can be used in a multi-column arrangement offering great savings when compared with a multi-pump arrangement for multi-column chromatography as described earlier[2].

DETAILS OF CONSTRUCTION

The new component in the multicolumn system that makes the use of a single pump possible in multi-column chromatography is the distributor shown in Fig. 1. It consists of a flat disc cut from cylindrical stainless-steel rod. A 1-in. hole is drilled half-way through the center of the disc and into this hole is threaded a Swagelok fitting that connects to the tubing coming from the pump. Channels are drilled from the peripheral ports located equidistant on the periphery of the distributor body to the central hole. Into each of these ports is fitted a needle valve (Hoke, No. 1325G2Y). This allows individual control of the solvent feed to each column so that as many or

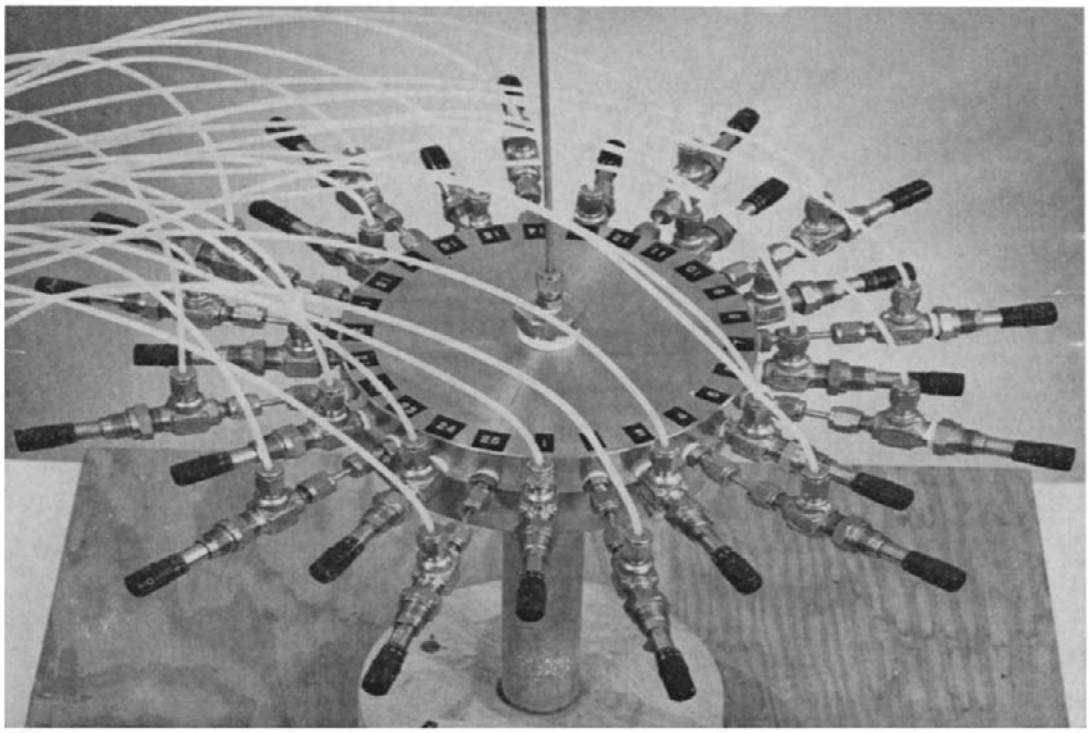

Fig. 1. A 25-valve distributor for multi-column chromatography. Liquid enters through the Swagelok fitting in the center and is from there distributed to 25 peripheral ports each fitted with a needle valve to permit operation of as few or as many valves as needed.

as few of the total number of columns as needed can be run at any time. The arrangement for multi-column chromatography using a single pump is shown in Fig. 2. In this case small volumes per hour were needed in the chromatography and one of the pump units in a duplex chromatographic minipump (Milton Roy, No. 196-89) was used to pump a gradient formed in a PTFE mixing bottle to the distributor and from there to a set of chromatographic columns. The second pump unit of the duplex pump is used to form the gradient. It pumps the more polar solvent from a measuring cylinder, allowing monitoring of flow per hour, into the less polar solvent in the mixing bottle.

A filter (Hoke, No. 6313F8S) is shown in the feeder line from the gradient bottle to the pump since it has been found important for the fail-safe performance of the system that no small particles enter the distributor. Although desirable also for the pump used for gradient formation it has been found possible to run this part without the filter. In the pump system higher capacity pumps than the one shown in Fig. 2 will usually have to be used to get enough flow. We have acquired a 9-l/h duplex diaphragm pump (Milton Roy, No. R-211A) for higher volume applications but even higher capacity pumps may be needed for some applications.

The complete system as it is currently used with compressed nitrogen as the pressure source is shown in Fig. 3 illustrating an eighteen-column system. The im-

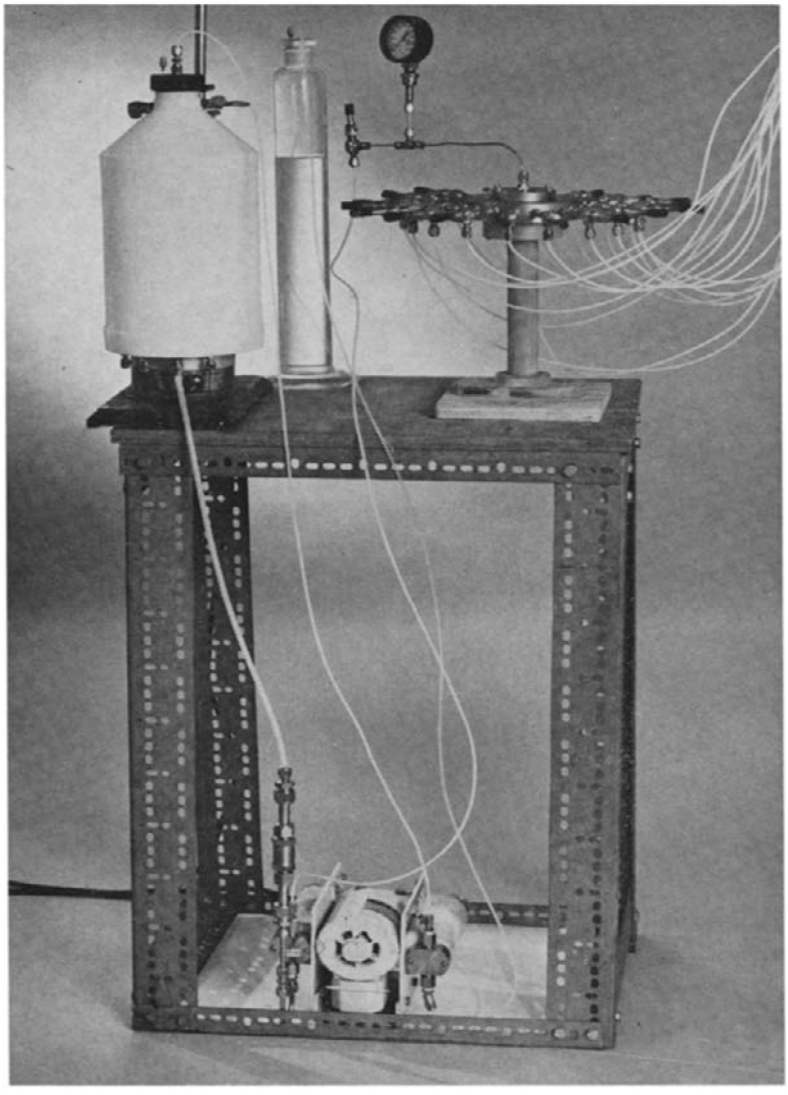

Fig. 2. Arrangement for single-pump multi-column chromatography. A gradient is formed in the PTFE mixing bottle to the left with stirring by a magnetic stirrer. The more polar solution is pumped from the graduated cylinder to the mixing bottle to form the gradient. The gradient eluent passes through a filter to the pump and is pumped to the distributor from which it goes to a set of columns.

portant changes in this system over the last few years are changes in the mixing-bottle design and the introduction of a cut-off valve controlled by a timer. The mixing bottle as currently used in the nitrogen pressure system is pictured in Fig. 4 together with the solenoid-operated timer-controlled three-way ball valve (Hoke, No. 7165G2Y) that makes it possible using an ordinary household timer to close off the connection to the nitrogen cylinder and open up to barometric pressure at any time during the 24 h so that for example a chromatographic run can be stopped during the night without

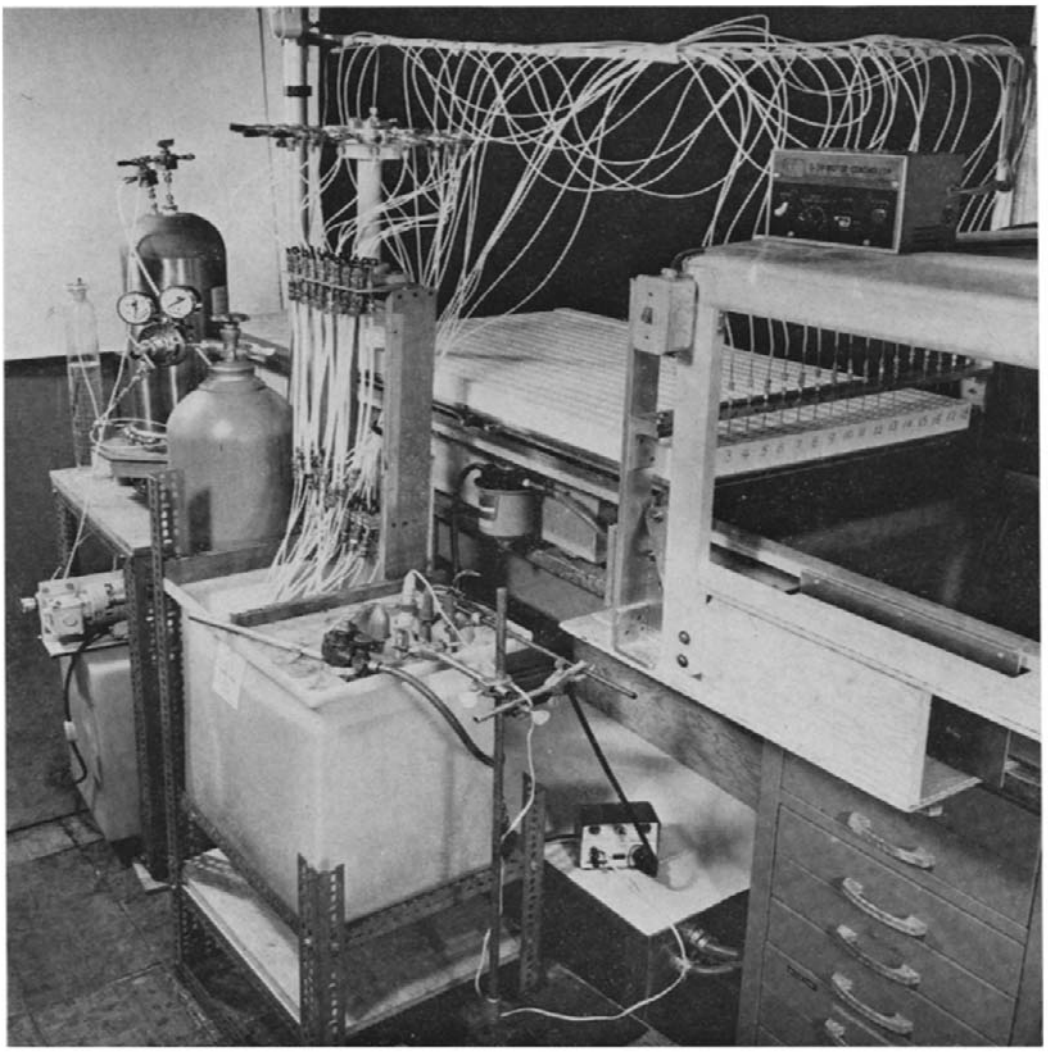

Fig. 3. The arrangement for 18-column chromatography using compressed nitrogen. The gradient for gradient elution chromatography is formed in the stainless-steel bottle in the back. The eluent is passed through the distributor positioned over the columns to the sample introduction section and from there to the capillary columns in the water-bath. The eluent from the columns goes to the multi-collector.

unnecessary loss of nitrogen when the mixing bottle otherwise would run empty —a considerable convenience factor.

The mixing bottle is an old twenty-five outlet bottle in which for economy reasons the old holes have been plugged up. Two outlets have been left. One connects to the distributor and through that to the chromatographic columns. The other allows easy drainage and wash-out of the bottle. One outlet and a three-way valve will serve both of these functions when new bottles are built. At the top of the bottle is seen the pressure inlet with its three-way valve for control of pressure and its tubing entering

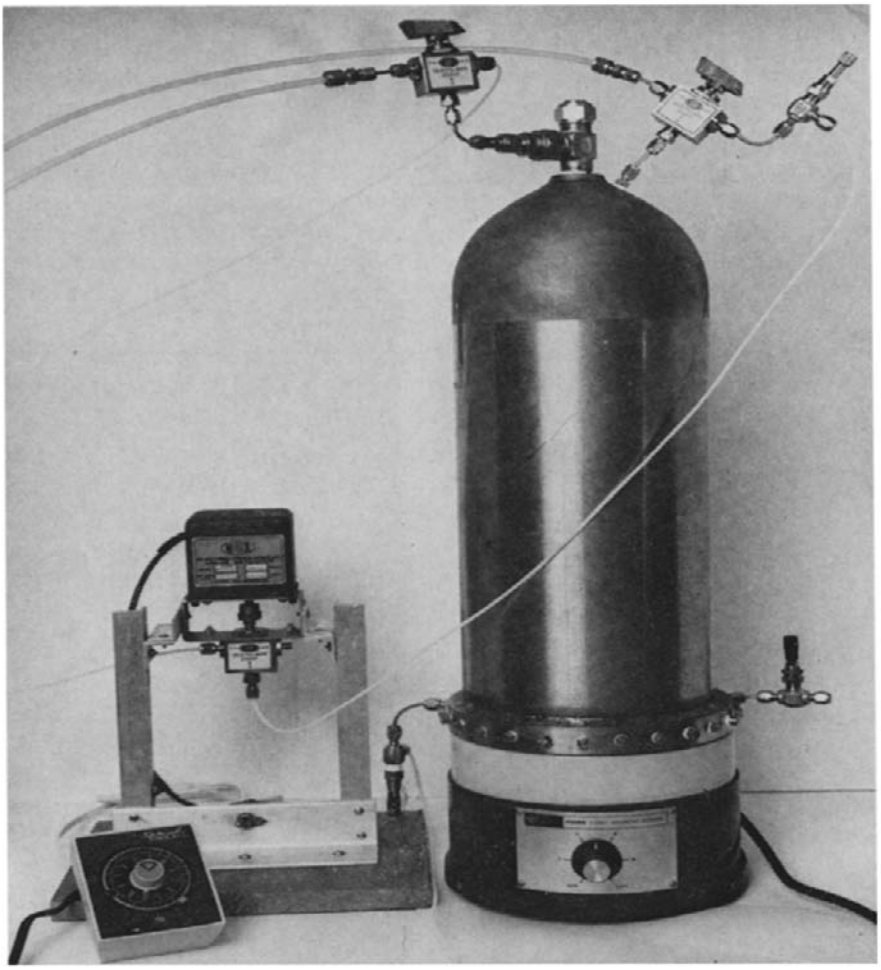

Fig. 4. The stainless-steel mixing bottle with three-way valves and inlets for pressure line and gradient solution at the top together with inspection plug at top of T-connector. Outlets at bottom connect to distributor and serve for emptying of bottle. A solenoid-activated three-way valve connects nitrogen pressure cylinder to mixing bottle and is controlled by a timer. The mixing bottle is placed on a magnetic stirrer.

the bottle off-center and also a large-diameter T-connector of stainless steel screwed into the central opening in the bottle. The gradient-producing solvent is carried through the side-arm and has its own three-way valve for control. The end of the T-connector has a removable plug through which the stirring magnet at the bottom of the mixing bottle is inspected before each run is started. For some applications we have previously found the use of nitrogen pressure systems superior in resolution to pump systems in multi-column chromatography[1]. This was, however, in chromatographic separations using organic solvents where samples were evaporated and then redissolved in a reagent mixture before quantitation. For other applications a pump system has been found preferable, for example in the chromatography of colored

compounds or colored derivatives that can be assayed directly after chromatography. It is important in this case that volumes are uniform from fraction to fraction and the nitrogen bubbles —so advantageous for improved resolution in the nitrogen pressure system compared with a pump system— are a drawback in this application since fractions are less uniform than in a pump system because of the irregularity of the bubble formation. The two systems thus supplement each other and give more versatility to the multi-column chromatographic system. The two new systems for multi-column chromatography share with our previously described system[1] the need for internal standards that must be added to the mixtures to be chromatographed since elution volumes, no matter how uniform the columns are in dimensions and how similarly they are packed, still vary somewhat from column to column.

With this provision, however, long practical experience with the systems in daily routine use has shown that both the nitrogen and pump systems are well suited for large-scale routine chromatography and this is clearly the main area of applicability for multi-column chromatographic systems.

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